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Importance of the Coombs test in diagnosing the Brucella

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Abstract

Aim: In Turkey and in many areas of the world, Brucellosis, which is a common zoonotic disease has been widely seen as the cause of serious economic loss. Serum samples from 100 patients, which were sent to the laboratory with a pre-diagnosis of Brucella, were examined at Gaziantep University Medicine Faculty Sahinbey Research Application Hospital. Wright and Coombs agglutination tests were performed on the serum samples.

Material and Methods: Patients were divided into three groups: Wright (+), Wright (-) & Coombs (+), and Wright (-) & Coombs (-). Biochemical parameters were evaluated to elucidate the correlation between the groups. Dilutions were examined with the Wright test and started at 1/20 titer to 1/2560 titer and the Coombs test was started at 1/80 titer to 1/640 titer.

Results: Our study showed that Wright test and Coombs test were found negative in 46 patients among 100, 11 patients among 100 exhibited a Wright (+) result, and 43 patients among 100 presented a Wright (-) and Coombs (+) result together. In biochemical analyzes, WBC was found to be significantly different ($p=0.019$) in Coombs (+) and Coombs (-) patients. There was a significant difference between the Wright (-) & Coombs (+) and Wright (+) groups in the GGT measurements ($p=0.038$). Based on the obtained data, the rate of the Wright (-) and Wright (-) & Coombs (+) patients were significantly higher (48.3%). In the serology assay, Brucella also appears to have a high frequency of blocking antibodies that cause false negative results with STA.

Discussion: Due to this high proportion of STA results, it is recommended to seek confirmation using other serological methods such as Coombs or ELISA. Therefore, patients who are admitted with a pre-diagnosis of Brucella should be examined by using the Coombs test or examination only with the Brucella Coombs test would be appropriate in order to avoid economic loss.

Keywords

Brucella, Coombs, Wright, Blocking Antibodies

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Introduction

Brucellosis is a common zoonotic disease around the world. It is commonly reported in humans and animals, especially in Mediterranean countries, the Arabian Peninsula, India, Africa and North America. Although it is controlled in developed countries, it remains a significant public health concern in developing countries [available at: http://cmuir.cmu.ac.th/handle/6653943832/35815]. According to data from the World Health Organization, 500.000 new cases are reported worldwide every year [1]. The disease occurs in people through consumption of unpasteurized milk and dairy products, inhalation of infected aerosols or direct contact with disintegrated tissues from animal secretions [2, 3]. Seroepidemiological studies have reported 9-25% and 3% seropositivity in occupational groups such as butchers, breeders, and slaughterhouse and dairy workers who were at risk of brucellosis and those who were not in the risk group, respectively [4, 5]. Showing intracellular settlement in humans and animals, the Brucella species are small, gram-negative, aerobic bacilli that cause infections [6]. In the serological diagnosis of brucellosis, IgG and IgM antibodies are studied with the Wright test, a Standard Tube Agglutination (SAT) test. However, while agglutinating antibodies in the structure of IgG, IgM and IgA occur in some individuals, blocking (non-agglutinating) or incomplete antibodies in the structure of IgG and IgA might occur in some other individuals [7-10]. The mechanism of this occurrence is not clearly understood. Blocking antibodies bind to antigens, however, agglutination does not occur. In the diagnosis of these incomplete antibodies, the Coombs test using anti-human immunoglobulin is applied [11].

No studies have been conducted on the prevalence of the formation of blocking antibodies in the regions of Turkey where brucellosis is particularly common. This study investigated the amount of blocking antibodies formed in these infections and potential differences that could occur between those developing blocking antibodies and those non-developing blocking antibodies in terms of biochemical parameters.

Material and Methods

Ethical approval for the present study was obtained from the local Clinical Research Ethics Committee (Date: 21.03.2016, Decision no: 2016/86). Serum from 100 patients who were referred to our laboratory with a pre-diagnosis of Brucella from various clinics at Gaziantep University Faculty of Medicine Sahinbey Research and Application Hospital were examined. Based on these criteria, 100 samples collected between April-December 2016 were studied as soon as they arrived, and the relevant results were recorded. Patients' age, gender and biochemical parameters were also recorded. Wright (Vircell, Spain) and Coombs (Vircell, Spain) agglutination tests were studied in the serum samples in line with the recommendations of the manufacturer [13]. In the patients who were Wright-negative, blocking antibodies were examined with the Coombs test. The Wright tube agglutination test was initiated at 1/20 titer and diluted up to titers of 1/2560. The Coombs test was initiated at 1/80 titer and diluted up to the titers of 1/640. Titers at and greater than 1/160 were considered positive in the Wright and Coombs agglutination tests.

Statistical analysis

The SPSS for Windows version 22.0 software package was used for statistical analysis and P<0.05 was considered statistically significant.

Ethical Approval

Ethics Committee approval for the study was obtained.

Results

Of the 100 patients evaluated in the study, 77 (77%) were female and 23 (23%) were male. According to the data obtained, there was no significant difference between the groups in terms of gender and mean age (Table 1).

In the study, positivity was detected via both methods in 54 (54%) of patients who were referred with a pre-diagnosis of Brucella. Only the Coombs test was found to be only positive in 43 (43%) patients. The Wright test and the Coombs test were negative, the Wright test was negative and the Coombs test was positive, and the Wright test was positive in 46 (46%) patients in group 1, 43 (43%) patients in group 2 and 11 (11%)

Table 1. Distribution of gender and age across the groups.

		Group 1 Wright (-) Coombs (-)		Group 2 Wright (-) Coombs (+)		Group 3 Wright (+)		
		Number	%	Number	%	Number	%	P
Gender	Male	13	28.3	8	18.6	2	18.2	0.514
	Female	33	71.7	35	81.4	9	81.8	
Age		40.41±18.65		41.23±16.52		41.36±19.71		0.972
Total		46		43		11		

Table 2. Distribution of study groups according to biochemical parameter results.

Variables	n	Group 1 Wright(-) Coombs(-)	n	Group 2 Wright(-) Coombs (+)	n	Group 3 Wright (+)	p
WBC	42	8.83±2.74	43	7.31±2.84	11	9.30±3.78	0.027
RBC	42	4.99±0.50	43	4.84±0.36	11	4.81±0.76	0.305
HGB	42	13.43±1.57	43	13.24±1.53	11	12.66±2.34	0.394
HCT	42	41.24±4.52	43	39.22±6.86	11	38.70±5.70	0.256
CRP	40	11.61±16.85	43	9.85±17.75	11	9.39±17.56	0.238
SEDIM	27	26.33±20.54	42	15.69±10.47	11	23.63±26.68	0.161
ALT	40	23.40±20.83	43	22.74±15.41	11	35.00±29.19	0.347
AST	27	25.37±14.84	41	25.65±12.50	11	30.54±18.17	0.491
GGT	42	46.00±0.00	30	22.86±12.43	7	54.28±43.46	0.038

Table 3. Statistical relationship between positive and negative groups in terms of biochemical parameters.

Biochemical parameters	Wright (-) Coombs (-) (n=42)	Wright or Coombs Positive (n=54)	P
WBC	8.83 ± 2.74	7.72 ± 3.12	0.072
RBC	4.99 ± 0.5	4.84 ± 0.46	0.124
HGB	13.43 ± 1.57	13.13 ± 1.72	0.378
HCT	41.25 ± 4.53	39.12 ± 6.6	0.077
CRP	11.61 ± 16.86	9.76 ± 17.55	0.609
SEDİM	26.33 ± 20.54	17.34 ± 15.3	0.051
ALT	23.4 ± 20.84	25.24 ± 19.34	0.660
AST	25.37 ± 14.85	26.69 ± 13.84	0.696
GGT	46.00±0.00	28.81 ± 24.4	0.880

patients in group 3 (Table 1).

When the biochemical parameters of the patients were examined, a significant difference was seen between Group1 and Group 2 only in WBC measurements (p=0.027). There is a significant difference between Group 3 and Group 1 (p=0.629). In GGT measurement, there was a significant difference between Group 2 and Group 3 (p=0.038). No significant difference in terms of biochemical parameters was found between the groups, which were negative in both parameters, i.e. Group 1, and the patients who were Wright- or Coombs-positive (Groups 2 and 3) (Table 2).

When the biochemical parameters were examined in the positive (Wright and/or Coombs) and negative groups, no significant difference was found.

Although no significant relationship was observed between the groups in terms of sedimentation, a biochemical parameter, the p-value obtained is notable.

Discussion

Brucellosis is a zoonotic disease caused by Brucella bacteria. According to statistics from the Turkish Public Health Association, the number of reported cases in Turkey in 2015 was 4.173. Its morbidity rate is 5.3/100.000 [available at: https://hsgmdestek.saglik.gov.tr/tr/zoonotikvektorel-bruselloz/istatistik]. Since its symptoms and findings are non-specific, it might imitate many other diseases. The definitive diagnosis method is by examination of the culture. However, bacteria can be cultured in approximately five days in blood culture and in seven days in bone marrow culture with automated systems. Serological methods are significant tests that are ancillary to the diagnosis of brucellosis. Among them, the Standard Tube Agglutination (STA) method is a test that is frequently used in Turkey. However, the presence of blocking antibodies complicates the diagnosis and reveals the need for the Coombs test. In this study, the extent of this need was sought to be determined. The difference in terms of biochemical parameters between the control group (Group 1) and other Brucella patients who developed blocking antibodies was also investigated.

Among the studies in Turkey, Gultekin [available at: https://tez.yok.gov.tr/UlusalTezMerkezi/tezDetay.jsp?id=UwQxrV_-pBJY700iGu1y9A&no=YCdDQaGPUa1wck3DQckHUQ] used four different methods in the postgraduate thesis that aimed to identify Brucella antibodies and covered 117 individuals with a pre-diagnosis of brucellosis. Of 85 patients with positivity in at least one of these tests, 57 (67.1%) and 28 (32.9%) were male and female, respectively. The age range of these patients was 5-75 (mean age 34.0) years. The RBT (Rose Bengal Test), STA, Coombs test and ELISA were used within the context of the study. The RBT, STA, CT and ELISA were positive in 81 (95.3%), 73 (85.9%), 64 (75.3%) and 67 (78.8%) of the serum samples, respectively. The study suggested that the RBT and STA were not always adequate in endemic regions to detect clinical features of the disease and to prevent cases that would adversely affect the diagnosis, such as, cross-reaction, the presence of blocking antibodies and a pre-existing presence, as well as preeclampsia follow up for low titers of STA. It has been suggested that laboratory diagnosis should be supported by tests such as Coombs and ELISA. In this study, 85 individuals were evaluated, and although the CT was 75%, blocking antibodies were not directly examined.

Age, gender, clinical symptoms and findings, routine laboratory results and the results of the Rose Bengal test, STA test and Coombs test of 50 patients who were diagnosed with brucellosis in 2011 were evaluated in a study by Altun et al. [12]. According to the results obtained, the mean age of the patients was 38.2 years, of which 62% and 38%, respectively were males and females (16±78), 50 of them were Rose Bengal-positive, the STA result was at and above 1/160 in 48 of them and 2 cases were Coombs-positive. No significant difference was found between age or gender and Brucella positivity. The study highlighted the importance of using Coombs serum while studying serums of patients suspected of having brucellosis and not agglutinating due to blocking antibodies. In this study, it was seen that Brucella positivity was not correlated with age and gender. However, there are studies asserting a correlation between Brucellosis and age and gender [13-15].

In the dissertation study from Canakkale, Ersoy [available at: https://acikbilim.yok.gov.tr/handle/20.500.12812/109207] found that 14 individuals who were negative in the STA test were Coombs-positive. Again in their study, Alıskan et al. demonstrated that the percentage of patients who were found to be positive using the STA test increased from 40% to 92% with the Coombs test.

Although there are studies showing that the amount of blocking antibodies can be very high in brucellosis, there are no studies investigating the seroprevalence of blocking antibodies in our region. This is why, our present study has important impact in related fields. One of the objectives of this study was to determine whether the presence of blocking antibodies was associated with biochemical parameters or not. However, no significant difference was found in the studies, except for GGT and WBC. GGT (gamma-glutamyl transferase) is an enzyme that is highly concentrated in the liver. The GGT value gives an indication about the health of the liver. The normal GGT value is accepted as 8-38 U/L. WBC (White Blood Cells) are produced in the bone marrow, lymph nodes, spleen and thymus gland. Their normal value interval is 3.98-10.04. A significant difference was found between Group 1 and Group 2 (p=0.019) in WBC measurements. However, this elevation is in favor of patients who were negative in both tests and may be associated with another disease rather than Brucellosis. While GGT values were normal in the Coombs-positive patients in the study, they were higher in the Wright-positive cases producing normal antibodies. A more extensive series of studies should investigate the reason why WBC and GGT are within normal limits in brucellosis cases with blocking antibodies. Sediment is a test that reveals acute phase reactants. In this study, although there was no significant relationship between Group 1 and Group 2 or Group 3 in terms of sedimentation, the results obtained are close to the significant difference value. Therefore, it is recommended to study the relationship between brucellosis patients and sedimentation as a biochemical parameter.

In our study, the Wright test was primarily applied to 100 serums of patients with a pre-diagnosis of brucellosis and the Coombs test was run on 89 negative samples. The Coombs test was positive in 43 Wright-positive samples (48.3%). This situation

is an important indicator of the extent to which patients, who present with a pre-diagnosis of Brucella and undergo only STA testing, cannot be diagnosed.

Conclusion

Present study shows that in the serology of Brucella, the frequency of blocking antibodies that cause false negative STA results is too high to be ignored (48.3%). Many studies recommend confirmation of STA results with other serological methods such as Coombs or ELISA. We are of the opinion that, due to this high rate, the Coombs test must also be studied in patients presenting with a pre-diagnosis of Brucella, or only the Brucella Coombs test should be studied to avoid economic loss.

Scientific Responsibility Statement
The authors declare that they are responsible for the article's scientific content including study design, data collection, analysis and interpretation, writing, some of the main line, or all of the preparation and scientific review of the contents and approval of the final version of the article.

Animal and human rights statement
All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. No animal or human studies were carried out by the authors for this article.

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Conflict of interest
None of the authors received any type of financial support that could be considered potential conflict of interest regarding the manuscript or its submission.

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